

Elischweski et al. — Appl. No. 09/680,310

I. AMENDMENT TO THE CLAIMS

Claims 1-28. (Cancelled).

Claim 29. (Currently Amended) A process for the preparation and improvement of a pantothenic acid-producing microorganism comprising amplifying a panE gene in said microorganism and then incubating said microorganism under conditions suitable for the production of the panE gene product, ketopantoate reductase wherein the microorganism is selected from the group consisting of *Escherichia* and *Saccharomyces*.

Claim 30. (Previously Presented) The process of claim 29, wherein said panE gene is overexpressed in said microorganism.

Claim 31. (Previously Presented) The process of claim 29, wherein the endogenous panE gene is amplified.

Claim 32. (Previously Presented) The process of claim 29, wherein the ilvC gene is additionally amplified.

Claim 33. (Previously Presented) The process of claim 30, wherein overexpression is achieved by insertion of a gene encoding a protein having ketopantoate reductase activity into a plasmid vector and then transforming said microorganism with said plasmid vector.

Claim 34. (Previously Presented) The process of claim 33, wherein a promoter is incorporated upstream of said gene encoding a protein having ketopantoate activity.

Claim 35. (Cancelled).

Claim 36. (Previously Presented) The process of claim 30, wherein overexpression is achieved by mutating a promoter or other regulatory element controlling coding sequence of a structural gene that prompts pantothenic acid production, the promoter or other regulatory element being located upstream of the structural gene.

Claim 37. (Currently Amended) The process of claim 29, wherein the gene that codes for ketopantoate reductase is amplified in a microorganism that has one or more metabolite

Elischweski et al. -- Appl. No. 09/680,310

resistance mutations wherein the microorganism is selected from the group consisting of *Escherichia* and *Saccharomyces*.

Claim 38. (Currently Amended) The process of claim 29, wherein the gene which codes for ketopantoate reductase is amplified in a microorganism, which has one or more antimetabolite resistance mutations wherein the microorganism is selected from the group consisting of *Escherichia* and *Saccharomyces*.

Claim 39. (Previously Presented) The process of either claim 37 or claim 38, wherein said microorganism is resistant to one or more compounds selected from the group consisting of: the metabolite L-valine; the metabolite alpha-ketoisovaleric acid; the antimetabolite salicylic acid; the antimetabolite alpha-ketobutyric acid; beta-hydroxyaspartic acid; and the antimetabolite O-methylthreonine.

Claim 40. (Previously Presented) The process of claim 29, wherein said microorganism over-expresses at least one protein selected from the group consisting of: the protein having ketopantoate reductase activity encoded by the panE gene of *Escherichia coli*; the protein encoded by the ilvC gene of *Corynebacterium glutamicum*; and the protein having ketopantoate reductase activity encoded by the YHR063c reading frame of *Saccharomyces cerevisiae*.

Claim 41. (Previously Presented) The process of claim 29, wherein, additionally, at least one gene of the metabolic path of pantothenic acid formation is amplified.

Claim 42. (Currently Amended) The process of claim 41, wherein said gene of the metabolic path of pantothenic formation is selected from the group consisting of: ketopantoate hydroxymethyltransferase (EC 4.1.2.12); aspartate 1-decarboxylase (EC 4.1.1.1); isomero reductase; and pantothenate synthetase (EC 6.3.2.1).

Claim 43. (Currently Amended) The process of claim 41, wherein said microorganism is transformed with a plasmid vector comprising at least one gene of the metabolic path of pantothenic acid formation selected from the group consisting of: ketopantoate hydroxymethyltransferase; aspartate 1-decarboxylase, isomero reductase, and pantothenate synthase.

Elischwesi et al. -- Appl. No. 09/680,310

Claim 44. (Previously Presented) The process of claim 29, wherein the activity of at least one gene in a metabolic pathway which reduces the formation of pantothenic acid is eliminated in said microorganism.

Claim 45. (Previously Presented) The process of claim 44, wherein the activity of the *avtA* gene is eliminated.

Claim 46. (Previously Presented) The process of claim 44, wherein the activity of the *ilvE* gene is eliminated.

Claim 47. (Previously Presented) The process of claim 29, wherein the *ilvC* gene of *C. glutamicum* is overexpressed or amplified in said microorganism.

Claim 48. (Cancelled).

Claim 49. (Cancelled).

Claim 50. (Currently Amended) The process of claim 49 29, wherein said microorganism is a bacterium of the species *Escherichia coli*.

Claim 51. (Cancelled).

Claim 52. (Cancelled).

Claim 53. (Cancelled).

Claim 54. (Currently Amended) The process of claim 53 29, wherein said microorganism is a yeast of is the species *Saccharomyces cerevisiae*.

Claim 55. (Previously Presented) The process of claim 44, wherein said microorganism is *Escherichia coli* and the activity of either the *avtA* or *ilvE* gene is eliminated in said microorganism.

Elischweski et al. -- Appl. No. 09/680,310

Claim 56. (Allowed) The plasmid vector pFE80 characterized by the restriction map shown in Figure 6 and deposited as *E. coli* K12 strain MG 1655/pFE80 under deposit number DSM 12414.

Claim 57. (Allowed) The plasmid vector pFE65, characterized by the restriction map shown in Figure 5 and deposited as *E. coli* K12 strain MG 1655/pFE65 under deposit number DSM 12382.

Claim 58. (Allowed) The plasmid vector pFE32, characterized by the restriction map shown in Figure 4 and deposited as *E. coli* K12 strain MG 1655/pFE32 under the deposit number DSM 12413.

Claim 59. (Previously Presented) The process of claim 29, wherein K12 strain FE6 is used, said strain being deposited under deposit number DSM 12379.

Claim 60. (Previously Presented) The process of claim 29, wherein *E. coli* K12 strain FE7 is used, said strain being deposited under deposit number DSM 12380.

Claim 61. (Currently Amended) The microorganism produced by the process of claim 29 wherein the microorganism is selected from the group consisting of *Eschericia* and *Saccharomyces*.